Version 14-04/24

User's Manual

Human Pancreatic Elastase ELISA Kit

REF **DEIA060J**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The described assay is intended for the quantitative determination of human pancreatic elastase in stool. For research use only.

General Description

Pancreatic elastase is an anionic endoprotease of the serine protease family with a molecular weight of 26 kDa. Together with other digestive enzymes it is synthesized as an inactive pro-enzyme in the acinar cells of the pancreas and is secreted into the duodenum. After its activation, pancreas elastase cleaves peptides after neutral amino acids.

Pancreas elastase is mainly bound to bile salts during intestinal passage and is not degraded. In human feces it is 5–6 fold more concentrated than in pancreatic juice. The stool concentration reflects the secretory capacity of the pancreas.

Indications:

Diagnosis/exclusion of exocrine pancreas in sufficiency in case of unexplained diarrhea, constipation, steatorrhea, flatulence, weightloss, upper abdominal pain, and food into lerances.

Monitoring of exocrine pancreas function in cystic fibrosis, diabetes mellitus, or chronic pancreatitis.

Principles of Testing

This ELISA is intended for the quantitative determination of pancreatic elastase in stool. In a first incubation step, the pancreatic elastase in the samples is bound to monoclonal antibodies, immobilized to the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a peroxidase-labeled conjugate (mouse anti pancreatic elastase) is added which recognizes specifically the bound pancreatic elastase. After another washing step to remove all unbound substances, the solid phase is incubated with the substrate, tetramethyl-benzidine (TMB), which reacts with the peroxidase. An acidic stop solution is added to stop the reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of pancreatic elastase. A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standards. Pancreatic elastase present in the patient samples is determined directly from this curve.

Reagents And Materials Provided

1. **PLATE** Microtiter plate, pre-coated, 12 x 8 wells
2. **WASHBUF** ELISA wash buffer concentrate, 10 x, 2 x 100 ml
3. **Extraction buffer concentrate**, 2.5 x, 1 x 100 ml
4. **CONJ** Conjugate concentrate (mouse anti pancreatic elastase), 1 x 200 µl
5. **STD** Standard, lyophilized, 4 x 5 vials
6. **CTRL1** Control, lyophilized, 4 x 1 vial

7. **CTRL2** Control, lyophilized, 4 x 1 vial
8. **SUB** TMB substrate (Tetramethylbenzidine), ready to use, 1 x 15 ml
9. **STOP** ELISA stop solution, ready to use, 1 x 15 ml

Materials Required But Not Supplied

1. Ultra pure water*
2. Calibrated precision pipettors and 10–1000 µl tips
3. Foil to cover the microtiter plate
4. Horizontal microtiter plate shaker
5. Multi-channel pipets or repeater pipets
6. Vortex
7. Standard laboratory glass or plastic vials, cups, etc.
8. Microtiter plate reader

* CD recommends the use of Ultra Pure Water (Water Type 1; ISO3696), which is free of undissolved and colloidal ions and organic molecules (free of particles>0.2µm) with an electrical conductivity of 0.055 µS/cm at 25°C(≥18.2MΩcm).

Storage

Store the kit at 2-8°C.

Specimen Collection And Preparation

Sample stability and storage

According to literature, the stability of pancreatic elastase in raw stool is 3 days at room temperature [5], 3 days at 4–8 °C [1], and up to a year at -20 °C [1].

Stool extract is stable at room temperature (15–30 °C) for three days, at 2–8 °C as well as at -20 °C for seven days. Avoid more than one freeze-thaw cycle.

Extraction of the stool samples

Diluted extraction buffer is used as a sample extraction buffer. We recommend the following sample preparation:

Stool Sample Application System (SAS)

Stool sample tube – Instructions for use

Please note that the dilution factor ofthe final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 1.5 ml extraction buffer:

Applied amount of stool: 15mg

Buffer Volume: 1.5ml

Dilution Factor: 1:100

Please follow the instructions for the preparation of stool samples using the SAS as follows:

a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.

b) Fill the empty sample tube with 1.5ml of ready to use extraction buffer before using it with the sample. **Important:** Allow the extraction buffer to reach room temperature.

c) Unscrew the tube (orange part of cap) to open. Insert the orange dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.

d) Shake the tube well until no stool sample remains in the notches. **Important:** Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with buffer for ~ 10 minutes improves the result.

e) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.

f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution I: 1:100

Dilution of samples

After centrifugation, the supernatant of the sample preparation procedure (dilution I) is diluted 1:100 in wash buffer. For this purpose, one of the two following dilution procedure variants can be used:

Variant A (recommended by CD):

• 100 µl supernatant (dilution I) + 900 µl wash buffer, mix well = 1:10 (dilution IIa)

• 100µl dilution IIa + 900µl wash buffer, mix well = 1:10 (dilution IIIa).

This results in a final dilution of 1:10 000.

For analysis, pipet 100 µl of dilution IIIa per well.

Variant B: Alternatively, the 1:100 dilution can be done in one step. For example:

• 10 µl supernatant (dilution I) + 990 µlwash buffer, mix well = 1:100 (dilution IIb). This results in a final dilution of 1:10 000.

For analysis, pipet 100 µl of dilution IIb per well.

Reagent Preparation

1. To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
2. Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
3. **Preparation of the wash buffer**: The wash buffer concentrate (WASHBUF) has to be diluted with ultra pure water **1:10** before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals could occur due to

high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or in a water bath at 37 °C before dilution of the buffer solutions. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. Wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8°C for one month**.

4. **Preparation of the extraction buffer:** The extraction buffer concentrate has to be diluted with ultra pure water **1:2.5** before use (100 ml extraction buffer concentrate + 150 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. Before dilution, the crystals must be redissolved at 37°C in a water bath. The **extraction buffer concentrate** is stable at **2– 8 °C** until the expiry date stated on the label. **Extraction buffer** (1:2.5 diluted extraction buffer concentrate) can be stored in a closed flask at **2–8°C for three months.**
5. The **lyophilized standards (STD)** and **controls (CTRL**) are stable at **2–8°C** until the expiry date stated on the label. Reconstitution details are given in the data sheet. Reconstituted standards and controls are not stable and **can not be stored**.
6. **Preparation of the conjugate:** Before use, the conjugate concentrate (CONJ) has to be diluted **1:101** in wash buffer (100 µl CONJ + 10 ml wash buffer). The **CONJ** is stable at **2–8°C** until expiry date stated on the label. **Conjugate**(1:101 diluted CONJ) is not stable and **can not be stored**.
7. All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2–8°C**.

Assay Procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark positions for **STD/SAMPLE/CTRL** (standard / sample / controls) in the protocol sheet. Take as many **microtiter strips** as needed from kit. Store unused strips covered at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISAprocessors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact CD. We recommend to carry out the tests in duplicate.

1. Add **100µl of STD/SAMPLE/CTRL** (standard / sample / controls) into respective well.
2. Cover the strips and incubate for **30 min** at room temperature (15-30 °C) on a horizontal shaker.
3. Discard the contents of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
4. Add **100 µl conjugate** to each well.
5. Cover the strips and incubate for **30 min** at room temperature (15-30 °C) on a horizontal shaker.
6. Discard the contents of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
7. Add **100 µl SUB** (TMB substrate) in each well.
8. Incubate for **10-20 minutes*** at room temperature (15-30 °C) in the dark.
9. Add **100 µl STOP** (ELISA stop solution) and mix well.
10. Determine **absorption immediately** with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm**

against 620 nm as a reference.

* The intensity of the color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.

Quality Control

CD recommends the use of external controls for internal quality control, if possible. Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality controlsample are outside the acceptable limits.

Calculation

The following algorithms can be used alternatively to calculate the results. We recommend using the "4 parameter algorithm".

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e. g. 0.001).

2. Point-to-pointcalculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration. The plausibility of the duplicate values should be examined before the automatic evaluation ofthe results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Stool samples

Multiply the result by the dilution factor of 10000 to obtain the concentration of pancreatic elastase in the sample. In case another dilution factor has been used, multiply the obtained result with the dilution factor used.

Reference Values

We recommend each laboratory to establish its own reference concentration range.

Reference range in stool samples[4]

1 g stool is equivalent to 1 ml.

> 200µg/ml normal value

100 - 200µg/ml slight to moderate exocrine pancreatic insufficiency

< 100µg/ml exocrine pancreatic insufficiency

Precision

Intra-Assay (n = 20)

Sample	Pancreatic elastase [µg/ml]	CV [%]
1	568.4	4.6
2	424.9	5.6

Inter-Assay (n = 12)

Sample	Pancreatic elastase [µg/ml]	CV [%]
1	449.1	7.7
2	379.7	9.2

Sensitivity

The LoB (limit of blank) was evaluated without considering sample dilution factor according to the guideline CLSI EP17-A2 and resulted in 0.66 µg/ml.

Specificity

No cross reactivity to the following proteins was observed:

• Pancreatic lipase

• Chymotrypsin

• Pankreatic amylase

• Pancreatin

• Calprotectin

Recovery

Spiking Recovery

Two samples were spiked with different pancreatic elastase concentrations and measured using this assay (n = 2).

Sample	Unspiked Sample [ng/ml]	Spike [ng/ml]	expected [ng/ml]	measured [ng/ml]
A	21.3	35.7	57.0	55.5
	21.3	26.6	47.9	42.4
	21.3	18.4	39.7	34.0
B	33.2	35.7	68.9	61.6
	33.2	26.6	59.8	51.0
	33.2	18.4	51.6	47.4

Dilution recovery

Three samples were diluted and analysed. The results are shown in the table below (n = 3):

Sample	Dilution	expected [µg/ml]	measured [µg/ml]
A	–	235.0	235.0
	1:2	117.5	115.0
	1:4	58.8	64.9
	1:8	29.4	36.3
B	–	171.0	171.0
	1:2	85.5	92.3
	1:4	42.8	49.4
	1:8	21.4	28.3
C	–	177.0	177.0
	1:2	88.5	90.1
	1:4	44.3	52.2
	1:8	22.1	27.4

Precautions

• All reagents in the kit package are for research use only.

• Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.

• Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin ormucous membranes.

• The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water.Do not breath vapour and avoid inhalation.

Technical hints

• Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.

• Control samples should be analyzed with each run.

• Reagents should not be used beyond the expiration date stated on kit label.

• Substrate solution should remain colourless until use.

• To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

• Avoid foaming when mixing reagents.

• Do not mix plugs and capsfrom different reagents.

• The assay should always be performed according the enclosed manual.

Limitations

Samples with concentrations above the measurement range must be further diluted and re-assayed. Please consider this greater dilution when calculatingtheresults.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

LoB × sample dilution factor to be used

Liquid stools may lead to false pancreatic elastase results. In such cases, we recommend to also consider clinical symptoms and other diagnostic tests for the final diagnosis and/or to request another patient sample.

References

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